METHODS OF DIAGNOSING RABIES WITH SPECIAL REFERENCE TO THE COMPLEMENT FIXATION TEST

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The diagnosis of rabies is a very important question in India where it is such a common disease among animals and where so many human beings are exposed to the infection every year. In the year 1912 — the last for which complete figures are available — 4,788 persons underwent anti-rabic treatment at the two Pasteur Institutes in India. The numbers treated have steadily increased each year since the Institutes were opened, but owing to the prejudices and ignorance of the people the figure given above probably by no means represents the total number of individuals actually bitten by rabid animals.

I will first give a short description of the present methods of diagnosing the disease. They are —

I. CLINICAL. In man there is rarely any doubt about the diagnosis once the disease has developed; there is the history of being bitten by a rabid animal usually somewhere between six weeks to three months before, though shorter incubation periods do occur. The disease starts with malaise and fever, but very soon the characteristic symptoms appear; they are an anxious terrified look, pain at the seat of the bite, convulsions, painful spasms of the organs of deglutition and respiration causing the symptom hydrophobia, going on to a condition of paralysis, death ensuing rapidly in 2 or 3 days. In the last case I saw death

occurred less than 24 hours after the first symptoms were noticed. The only conditions that may be confused with rabies are, Lyssophobia in which there are hysterical manifestations, tetanus and mania, but the history and course of the case enable these diseases to be readily distinguished.

In animals too the symptoms as a rule are distinctive, and are divided into furious and paralytic. The first thing usually noticed is that the animal looks sick and refuses food, and such an animal should always be tied up and looked on with suspicion in a country where rabies is common. There soon is a change in the demeanour of the animal and it becomes restless and in furious cases bites at everything and everybody near it. There is usually a very characteristic change in the bark, which has been described, as like the belling of a tired hound, and saliva dribbles from the mouth. Paralysis soon supervenes, weakness of the hind legs being as a rule first noticed, gradually getting worse until the animal cannot stand. The paralysis quickly extends, until it affects the respiratory centres and death takes place. The total duration of the disease rarely lasts for more than three to four days.

While this is the typical picture certain cases are paralytic from the outset, and in others the symptoms may be so mild as to hardly rouse suspicion. I have seen a dog in which the only syptoms were some strangeness in behaviour for a few days and fits, which closely resembled epilepsy.

It did not look very ill and took its food; 24 hours later it was found dead in the kennel and examination of its brain showed that it had died of rabies. It is in such cases that help may be got from the laboratory methods of diagnosis, which are described below.

II. MICROSCOPICAL. i.e. the demonstration of Negri bodies in the Hippocampus major or Cerebellum. Negri bodies can be seen in fresh or stained smears of these tissues, but this method is often impracticable in India, where specimens have to be sent a long way to the laboratory often taking two or more days on the way, so that in the majority of cases it is necessary to cut and stain sections which takes another 2 or 3 days. Therefore it frequently happens that a week elapses from the time of the despatch of the brain, until a definite opinion can be given on the laboratory findings. This may involve serious delay in commencing antirabic treatment, when, as sometimes happens, patients wait for the result before starting for a Pasteur Institute.

Then there is the question of the specificity of the test. It is now generally agreed that the finding of Negri bodies is definite evidence of rabies. They are easily demonstrable after a little experience by anyone accustomed to Pathological technique. They are usually present in considerable numbers and it is comparatively seldom that more than one section has to be examined in order to find them. However the absence of Negri bodies cannot be taken as definite evidence that the brain is not a rabid one and it is not by itself sufficient to negative treatment, for it is

found that they are absent, or at least not demonstrable, in a small proportion of cases of rabies. Most authorities state that this occurs in from 2 to 5 % of cases. In this Institute last year out of 132 pieces of suspected tissue examined Negri bodies could not be found in two cases, which a biological test subsequently proved to be specimens of rabid brains.

Also it has been shown (1) in some work recently done in this Institute that Negri bodies may only become demonstrable after one or more subpassages. In one experiment described in that paper a series of 9 dogs were inoculated subdurally with a street virus in its 1st subpassage. All developed typical symptoms of rabies within 12 days, and yet the most careful examination of their brains failed to reveal the presence of Negri bodies.

In addition to this lack of specificity under the most favourable conditions we have the fact that many of the brains that come to a Pasteur Institute for diagnosis are sent up by people entirely ignorant of Pathological technique, so that they are frequently broken up, putrid or otherwise unfit for examination. Also there is a common practice of destroying an animal simply because it has bitten a person. In such cases Negri bodies may not be present and yet the saliva may be virulent, if the animal is in the presymptomatic or very early stage of rabies. Other microscopical changes in rabid brains have been described by Babes, Golgi and Van Gehuchten, (2) but I have no practical experience of them, and as methods of

diagnosis of rabies they have been entirely superseded by the search for Negri bodies.

III. BIOLOGICAL. This means the subdural inoculation of a small quantity of the suspected brain into a test animal, rabbits or guineapigs being generally used for this purpose. For this test it is necessary to have the brain fresh or preserved in glycerine. If the brain is a rabid one the test animals will, in the vast majority of cases, show symptoms of rabies between the 8th and 20th days, but in order to negative rabies it is necessary to keep them under observation for at least 2 months. In the last 9 biological tests done here the incubation periods were 9,11, 11,9,26,14,10,22 and 10 days respectively.

In most Pasteur Institutes it is now the practice to resort to the biological test in all cases of negative Negri body findings; on account of the time it takes this test is of no help in deciding on the necessity of anti-rabic treatment in doubtful cases; apart from this the results of the tests are satisfactory and it is of great value in confirming a diagnosis and in experimental work.

From the foregoing considerations it will be seen that the present laboratory methods for the diagnosis of rabies have each got certain limitations from a practical point of view, and it would be a very great advantage if an absolutely reliable test, or at least one that could be relied on as confirmatory of negative Negri body findings could be devised. Cases occur in which no proper history of the biting animal is obtainable and also cases in which

the biting animal is destroyed before any symptoms have developed. It is in such cases that a certain laboratory test would be of very great value in deciding whether it is necessary for the bitten persons to undergo antirabic treatment or not. It is in suspected rabid animals that the laboratory tests are of such importance; cases of people exposed to infection from human beings are rare and even then there is hardly ever any reasonable doubt as to the diagnosis.

THE COMPLEMENT FIXATION TEST IN RABIES.

This test has come into such general use as an aid in the diagnosis of certain diseases and especially in syphilis, that it seemed reasonable to suppose that if a suitable technique could be devised, it might prove of value in rabies also, and it was with this object in view that the experiments described below were undertaken. Such a serum test would have certain advantages over the present methods, namely —

It is easier and also less dangerous to collect a sample of blood than to dissect out a brain; it would not deteriorate in the same way as it could always be reactivated. It would come up quicker as it could be sent by post and the results of the test could be given earlier.

I do not intend to enter into any theoretical considerations of the subject of complement fixation. The technique adopted will be first described, then the experiments will be given in detail in the form of tables and lastly a brief summary of the results and conclusions will be given.

TECHNIQUE.

(a) Antigen.- Several antigens have been used. Emulsions of normal brain, Fixed virus brain, Street virus brain, Carbolized normal and Fixed virus brain. The fixed virus brains were obtained from the ordinary passage rabbits. The street virus brains used were got from a 1st subpassage in a guineapig of a dog's brain, which showed numerous Negri bodies. In all cases an equal number of tubes were put up with normal brain emulsion as a control. An emulsion of 1 in 50 was made in normal saline and then filtered through filter paper. The amount used was 1 cc. (in one experiment .5 cc.).

In the experiments in which carbolized brain (fixed virus and normal) was used as antigen it was 1 cc of a 1 in 50 emulsion of brain in .5 % Carbolic acid.

In a single instance an emulsion of submaxillary gland was used as antigen. This was obtained from a dog experimentally infected with street virus.

(b) Immune serum.- This was obtained from a horse that has had numerous injections of Fixed virus brain emulsion for the last four years. The injections were given at irregular intervals, at first daily or almost so for a fortnight, and then a fortnight's interval before another

course was given — the dose varied from .4 grm brain substance to 1 grm.

Later weekly injections of .8 to 1 grm. brain substance were given and lastly monthly injections of doses of 1 to 2 grms. of brain substance.

Some experiments were also done with the serum obtained from rabid dogs. These dogs were infected experimentally and it was proved by microscopic examination and subpassage tests that they died of rabies.

When the symptoms of rabies were very marked the dog was chloroformed and while under chloroform and before death a considerable amount of blood was taken from the heart, and the serum collected the next day.

(c) The Haemolytic system (sheep V goat).

Consisted of serum from a sheep that had had numerous injections of goat's corpuscles for about 10 months previous to the experiments; a 5 % suspension of goat's washed corpuscles (washed five times) and fresh guineapig's serum as complement.

The method of the test was carried out as follows:- 1 cc of the brain emulsion was pipetted into a test tube and to this was added .5 cc of the Immune serum which had been previously heated three times to 55°C for half an hour; the mixture was placed in the incubator at 37°C for one and a half hours. Then it was centrifuged and the deposit was taken and washed once with normal saline solution; to this was then added normal guinea-pig's serum in varying amounts as complement and sufficient saline solution to make the

amount of fluid in each tube the same. It was again incubated for one and a half hours and afterwards the haemolytic system was added, i.e., .4 cc sheep serum which had been heated to 55°C for half an hour, .5 cc of a 5 % suspension of goat's washed corpuscles and lastly sufficient saline solution to make up the total volume in each tube to 2 cc. The tubes were again incubated at 37°C for an hour and a half and placed in the ice chest until next morning, when the readings were made.

Preliminary tests were made to discover the minimum haemolytic doses of both complement and immune body.

The minimum haemolytic dose of complement was .055 and of immune body .2.

The following list gives the abreviations used in the tables with their meanings:-

N.B.	Normal brain emulsion $\frac{1}{50}$
F.V.	Fixed virus brain emulsion $\frac{1}{50}$
C.N.B.	Carbolized normal brain emulsion $\frac{1}{50}$
C.V.B.	Carbolized Fixed virus " "
S.V.B.	Street virus brain (guinea-pig).
N.B.G.P.	Normal brain (guinea-pig).
I.H.S.	Immune horse serum heated to 55°
R.D.S.	Rabid dog's serum 55°.
Dog II & III.	Rabid dog's serum 55°.
G.P.S.	Fresh normal guinea-pig's serum.
Sal.	Normal saline.
Sheep S serum.	Serum of sheep which had an Immune body for goat's corpuscles.

W.G.C.	Washed goat's corpuscles.
≋	= Centrifuge five minutes.
/	= Incubate at 37° for one and a half hours.
//	= 24 hours at room temperature.
E.S.M.G.	Emulsion of submaxillary gland.
C.	Complete haemolysis 100 %.
N.Q.C.	Not quite complete haemolysis - over 90 %
A.C.	Almost complete haemolysis, approximately 70 to 90 %.
V.M.	Very marked haemolysis, approximately 50 to 70 %.
M.	Marked haemolysis, approximately 30 to 50 %.
S.	Slight haemolysis, approximately 20 to 30 %.
T.	Trace of haemolysis, approximately 10 to 20 %.
S.T.	Slight trace of haemolysis, less than 10 %.
N.	No haemolysis.

No. I

.B. $\frac{1}{50}$	F.V.B. $\frac{1}{50}$	I.H.S.55	G.P.S.	Sal.	Sheep S serum.	W.G.C.	Sal.	Result.
.5	...	...	.075	.425	.4	.5	.1	C
.5	...	...	.1	.4	.4	.5	.1	C
.5	...	...	.125	.375	.4	.5	.1	C
.5	...	...	.15	.35	.4	.5	.1	C
.5	...	.5	.075	.425	.4	.5	.1	C
.5	...	.5	.1	.4	.4	.5	.1	C
.5	...	.5	.125	.375	.4	.5	.1	C
.5	...	.5	.15	.35	.4	.5	.1	C
...	.5	.5	.075	.425	.4	.5	.1	C
...	.5	.5	.1	.4	.4	.5	.1	NQ C
...	.5	.5	.125	.375	.4	.5	.1	C
...	.5	.5	.15	.35	.4	.5	.1	NQ C
...	...	.5	...	...	...	.5	.1	N
...	...	...	.1	...	...	.5	1.4	T
...	...	...	.075	...	.4	.5	1.075	C
...	...	...	.1	...	...	.5	1.0	C
...	...	....	.125	...	.4	.5	.975	C
...	...	...	.15	...	.4	.5	.95	C
...	...	...	...	...	.4	.5	1.1	N
.5	...	...	...	...	...	.5	1.0	N
...	.5	...	...	...	...	.5	1.0	N
...	...	.5	.1	...	.4	.5	.5	C

Immune horse serum + Fixed virus brain $\frac{1}{50}$ deviated a slight amount of complement as compared with normal brain. i.e., all the tubes put up with normal brain gave complete haemolysis, while two out of the four tubes put up with Fixed virus brain showed incomplete haemolysis. The result however was not a marked one.

No. II

N.B. 1/50	F.V.B. 1/50	I.H.S.55	G.P.S.	Sal.	Sheep S serum.	W.G.C.	Sal.	Result.
1	...	...	.025	.274	.4	.5	.3	N
1	...	...	.05	.25	.4	.5	.3	S
1	...	...	.075	.225	.4	.5	.3	M
1	...	...	.1	.2	.4	.5	.3	AC
1	...	.5	.025	.275	.4	.5	.3	N
1	...	.5	.05	.25	,4	.5	.3	S
1	...	.5	.075	.225	.4	.5	.3	M
1	...	.5	.1	.2	.4	.5	.3	AC
...	1	.5	.025	.275	.4	.5	.3	N
...	1	.5	.05	.25	.4	..5	.3	N
...	1	.5	.075	.225	.4	.5	.3	N
...	1	.5	.1	.2	.4	.5	.3	N

This experiment showed considerable deviation of complement by **Fixed** virus brain and Immune horse serum. i.e., there was no haemolysis in all these tubes while in the tubes with normal brain and Immune Horse Serum there was marked, but not complete haemolysis. Unfortunately in this instance no control tubes were put up with Fixed virus brain without horse serum.

No. III

N.B. 1/50	F.V.B. 1/50	I.H.S.55	G.P.S.	Sal.	Sheep S serum.	W.G.C.	Sal.	Result.
1	...	...	.025	.275	.4	.5	.3	N
1	...	...	.05	.25	.4	.5	.3	T
1	...	...	.075	.225	.4	.5	.3	S
1	...	...	.1	.2	.4	.5	.3	VM
1	...	.5	.025	.275	.4	.5	.3	N
1	...	.5	.05	.25	.4	.5	.3	N
1	...	.5	.075	.225	.4	.5	.3	N
1	...	.5	.1	.2	.4	.5	.3	S
...	1	...	.025	.275	.4	.5	.3	N
...	1	...	.05	.25	.4	.5	.3	N
...	1	...	.075	.225	.4	.5	.3	S
...	1	...	.1	.2	.4	.5	.3	VM
...	1	.5	.025	.275	.4	.5	.3	N
...	1	.5	.05	.25	.4	.5	.3	N
...	1	.5	.075	.225	.4	.5	.3	N
...	1	.5	.1	.2	.4	.5	.3	S
...	...	...	.1	...	...	.5	.9	N
...	...	.5	.1	...	.4	.5	...	NQC
...	...	...	.05	...	.4	.5	.55	NQC
...	...	...	.06	...	.4	.5	.54	C

This experiment which was completely controlled showed a similar inhibition of haemolysis with both Fixed virus and normal brain.

No. IV.

N.B	F.V.B	C.F.V	I.H.S	G.P.S	Sal.	Sheep S.	W.G.C.	Sal.	Result.
1	...	...	...	.05	.25	.4	.5	.3	S
1	...	...	...	.075	.225	.4	.5	.3	AC
1	...	...	...	.1	.2	.4	.5	.3	C
1	...	...	....	.125	.175	.4	.5	.3	C
1	...	...	.5	.05	.25	.4	.5	.3	S
1	...	...	.5	.075	.225	.4	.5	.3	M
1	...	...	.5	.1	.2	.4	.5	.3	C
1	...	...	.5	.125	.175	.4	.5	.3	C
...	1	...	...	.05	.25	.4	.5	.3	S
...	1	...	...	.075	.225	.4	.5	.3	AC
...	1	...	...	.1	.2	.4	.5	.3	C
...	1	...	...	.125	.175	.4	.5	.3	C
...	1	...	.5	.05	.25	.4	.5	.3	N
...	1	...	.5	.075	.225	.4	.5	.3	T
...	1	...	.5	.1	.2	.4	.5	.3	S
...	1	...	.5	.125	.175	.4	.5	.3	VM
...	...	1	...	.05	.25	.4	.5	.3	VM
...	...	1	...	.075	.225	.4	.5	.3	AC
...	...	1	...	.1	.2	.4	.5	.3	C
...	...	1	...	.125	.175	.4	.5	.3	C
...	...	1	.5	.05	.25	.4	.5	.3	N
...	...	1	.5	.075	.225	.4	.5	.3	N
...	...	1	.5	.1	.2	.4	.5	.3	AC
...	...	1	.5	.125	.175	.4	.5	.3	C
...	...	...	.5	...	...	...	.5	.5	N
...	...	....	.5	.05	...	...	.5	.45	T

In this case 8 tubes were also put up with carbolized Fixed virus brain as antigen: the result was apparent slight deviation of complement both with Fixed virus brain and carbolized Fixed virus brain as compared with normal brain.

No. V.

N B.	C F B.	N.B	F.V	I H S.	G P S.	Sal.	Sheep S.	W G C.	Sal.	Result.
1	...	...	...	...	.055	.245	.4	.5	.3	M
1	...	...	...	...	.11	.19	.4	.5	.3	NQC
1	...	...	...	...	.165	.135	.4	.5	.3	C
1	...	...	...	.5	.055	.245	.4	.5	.3	S
1	...	...	...	.5	.11	.19	.4	.5	.3	NQ
1	...	...	...	.5	.165	.135	.4	.5	.3	C
..	1	...	...	...	.055	.245	.4	.5	.3	VM
..	1	...	...	...	.11	.19	.4	.5	.3	NQC
..	1	...	...	...	.165	.135	.4	.5	.3	C
..	1	...	....	.5	.055	.245	.4	.5	.3	T
..	1	...	...	.5	.11	.19	.4	.5	.3	VM
..	1	...	...	.5	.165	.135	.4	.5	.3	NQC
..	...	1	...	...	.055	.245	.4	.5	.3	M
..	...	1	...	...	.11	.19	.4	.5	.3	C
..	...	1	...	...	.165	.135	.4	.5	.3	C
..	...	1	...	.5	.055	.245	.4	.5	.3	S
..	....	1	...	.5	.11	.19	.4	.5	.3	AC
..	...	1	...	.5	.165	.135	.4	.5	.3	C
..	...	...	1	...	.055	.245	.4	.5	.3	M
..	...	...	1	...	.11	.19	.4	.5	.3	C
..	...	...	1	...	.165	.135	.4	.5	.3	C
..	...	...	1	.5	.055	.245	.4	.5	.3	S
..	...	...	1	.5	.11	.19	.4	.5	.3	VM
..	...	...	1	.5	.165	.135	.4	.5	.3	NQC
..	...	...	...	.5	.055	...	.4	.5	...	VM
..	...	...	...	...	...	...	.4	.5	.6	N

Fixed virus brain carbolized and fresh deviated slightly more complement than normal brain carbolized and fresh, but the result was in no degree marked.

No. VI

TB	CFV.	N.B	F.V	G.Pig S.V.B	I HS.	G P S.	Sal.	Sheep S	W.G.C	Sal.	Result.
.	...	1	...	...	....	.055	.245	.4	.5	.3	S
.	...	1	...	...	...	.11	.19	.4	.5	.3	AC
.	...	1	...	...	,5	.055	.245	.4	.5	.3	T
.	...	1	...	...	.5	.11	.19	.4	.5	.3	S
.	...	...	1	...	...	.055	.245	.4	.5	.3	VM
.	...	...	1	...	...	.11	.19	.4	.5	.3	NQC
.	...	...	1	...	.5	.055	.245	.4	.5	.3	T
.	...	...	1	...	.5	.11	.19	.4	.5	.3	S
.	...	...	...	1	...	.055	.245	.4	.5	.3	S
.	...	...	...	1	...	.11	.19	.4	.5	.3	AC
.	...	...	...	1	.5	.055	.245	.4	.5	.3	N
.	...	...	...	1	.5	.11	.19	.4	.5	.3	T
.	...	1	...	...	...	...	...	...	.5	1.0	N
.	...	...	1	...	...	...	...	...	.5	1.0	C
.	...	...	...	1	...	...	...	...	.5	1.0	N
.	...	1	...	...	...	...	...	.4	.5	.6	N
.	...	...	1	...	...	...	...	.4	.5	.6	N
.	...	...	...	1	...	...	...	.4	.5	.6	N
.	...	...	...	...	.5	...	...	...	.5	.5	ST
	...	...	...	...	...	...	...	...	.5	1.0	N
.	1	...	...	...	...	...	...	...	.5	1.0	N
.	...	...	...	...	...	.055	...	.4	.5	.545	C

Street virus brain showed slightly more deviation with horse serum than did either Fixed virus or normal brain, but all gave a certain amount of deviation.

No. VII.

N.B	F.V.B	.G.P N.B	G.P S.V.B	I.H.S	G.P.S	Sal.	Sheep S	W.G.C	Sal.	Result.
1	...	...	...	...	.055	.245	.4	.5	.3	T
1	...	...	...	...	.11	.19	.4	.5	.3	VM
1	...	...	...	.5	.055	.245	.4	.5	.3	N
1	...	...	...	.5	.11	.19	.4	.5	.3	T
...	1	...	...	...	.055	.245	.4	.5	.3	N
...	1	...	...	....	.11	.19	.4	.5	.3	S
...	1	...	...	.5	.055	.245	.4	.5	.3	N
...	1	...	...	.5	.11	.19	.4	.5	.3	S
...	...	1	...	...	.055	.245	.4	.5	.3	S
...	...	1	...	...	.11	.19	.4	.5	.3	AC
...	...	1	...	.5	.055	.245	.4	.5	.3	T
...	...	1	...	.5	.11	.19	.4	.5	.3	VM
...	...	...	1	...	.055	.245	.4	.5	.3	N
...	...	...	1	...	.11	.19	.4	.5	.3	ST
...	...	...	1	.5	.055	.245	.4	.5	.3	ST
...	...	...	1	.5	.11	.19	.4	.5	.3	S
...	...	...	...	.5	...	...	...	.5	.5	N

Here normal brain and immune horse serum absorbed slightly more complement than Fixed virus brain and immune horse serum. Fixed virus brain alone absorbed as much complement as Fixed virus brain and immune horse serum. Street virus brain alone showed slightly more absorption of complement than street virus brain + Immune horse serum. There is nothing in this experiment to indicate that the horse serum possessed an Immune body for either Fixed virus or Street virus.

No. VIII.

N.B	F.V.B	I.H.S	G.P.S	Sal.	Sheep S.	W.G.C	Sal.	Result.
1	...	...	.055	.245	.4	.5	.3	N
1	...	...	.11	.19	.4	.5	.3	N
1	...	.5	.055	.245	.4	.5	.3	N
1	...	.5	.11	.19	.4	.5	.3	N
...	1	...	.055	.245	.4	.5	.3	N
...	1	...	.11	.19	.4	.5	.3	T
...	1	.5	.055	.245	.4	.5	.3	N
...	1	.5	.11	.19	.4	.5	.3	N
...	...	.5	...	...	.4	.5	.1	T
...	...	.5	.055	...	...	.5	.445	S

Here, normal brain by itself absorbed all the complement and fixed virus brain absorbed practically all. The Horse serum alone also absorbed a certain amount of complement. The working of the haemolytic system was not controlled.

No. IX

N.B	F.V.B	R.D.S Dog II.	G.P.S	Sal.	Sheep S	W.G.C	Sal.	Result.
1	...	...	.025	.275	.4	.5	.3	N
1	...	...	.05	.25	.4	.5	.3	T
1	...	...	.075	.225	.4	.5	.3	S
1	...	...	.1	.2	.4	.5	.3	VM
1	...	.5	.025	.275	.4	.5	.3	N
1	...	.5	.05	.25	.4	.5	.3	N
1	...	.5	.075	.225	.4	.5	.3	Tube accidentally broken
1	...	.5	.1	.2	.4	.5	.3	-do-
...	1	...	.025	.275	.4	.5	.3	N
...	1	...	.05	.25	.4	.5	.3	N
...	1	...	.075	.225	.4	.5	.3	S
...	1	...	.1	.2	.4	.5	.3	VM
...	1	.5	.025	.275	.4	.5	.3	N
...	1	.5	.05	.25	.4	.5	.3	N
...	1	.5	.075	.225	.4	.5	.3	N
...	1	.5	.1	.2	.4	.5	.3	N
...	...	.5	...	...	...	.5	.5	N
...	...	.5	...	...	.4	.5	.1	T
...	...	.5	.1	...	.4	.5	...	C *
...	...	...	.06	...	.4	.5	.54	C

The result was that rabid dog's serum appeared to deviate complement both with Fixed virus brain and normal brain. There was no haemolysis in the tubes with rabid dog's serum while in the tubes without rabid dog's serum, but with antigen, complement and the haemolytic system there was considerable haemolysis.

No. X

N.B	F.V	C.N.B	C.F.B	R.D.S Dog II.	G.P.S	Sal.	Sheep S	W.G.C	Sal.	Result.
1	...	...	...	...	.055	.245	.4	.5	.3	C
1	...	...	...	...	.11	.19	.4	.5	.3	C
1	...	...	...	.5	.055	.245	.4	.5	.3	N
1	...	...	...	.5	.11	.19	.4	.5	.3	N
...	1	...	...	...	.055	.245	.4	.5	.3	C
...	1	...	...	...	.11	.19	.4	.5	.3	C
...	1	...	...	.5	.055	.245	.4	.5	.3	N
...	1	...	...	.5	.11	.19	.4	.5	.3	N
...	...	1	...	...	.055	.245	.4	.5	.3	C
...	...	1	...	...	.11	.19	.4	.5	.3	C
...	...	1	...	.5	.055	.245	.4	.5	.3	N
...	...	1	...	.5	.11	.19	.4	.5	.3	N
...	...	...	1	...	.055	.245	.4	.5	.3	NQC
...	...	...	1	...	.11	.19	.4	.5	.3	C
...	...	...	1	.5	.055	.245	.4	.5	.3	N
...	...	...	1	.5	.11	.19	.4	.5	.3	N
...	...	...	....	.5	...	...	.4	.5	.5	N
...	...	...	...	.5	.055	...	.4	.5	.045	VM
...	...	...	....	.5	.11	...	.4	.5	...	C
...	...	...	...	...	.055	...	.4	.5	.545	C
...	...	1	...	...	...	...	...	.5	...	S

In this case the antigens used were normal brain, Fixed virus brain, Carbolized normal brain and Carbolized Fixed virus brain. With all these there was complete inhibition of haemolysis showing that antigen + rabid dog's serum had absorbed complement. The control tubes without dog's serum show complete haemolysis.

No. XI

.B	F.V	C.N.B	C.F.B	R.D.S Dog.III	G.P.S	Sal.	Sheep S	W.G.C	Sal.	Result.
1	...	...	...	...	.055	.245	.4	.5	.3	S
1	...	...	...	...	.11	.19	.4	.5	.3	M
1	...	...	....	.5	.055	.245	.4	.5	.3	N
1	...	...	...	.5	.11	.19	.4	.5	.3	T
..	1	...	...	...	.055	.245	.4	.5	.3	S
..	1	...	...	...	.11	.19	.4	.5	.3	M
..	1	...	...	.5	.055	.245	.4	.5	.3	N
..	1	...	...	.5	.11	.19	.4	.5	.3	T
..	...	1	...	...	.055	.245	.4	.5	.3	S
..	...	1	...	...	.11	.19	.4	.5	.3	VM
..	...	1	...	.5	.055	.245	.4	.5	.3	S
..	...	1	...	.5	.11	.19	.4	.5	.3	VM
..	...	...	1	...	.055	.245	.4	.5	.3	S
..	...	...	1	...	.11	.19	.4	.5	.3	VM
..	...	...	1	.5	.055	.245	.4	.5	.3	S
..	...	...	1	.5	.11	.19	.4	.5	.3	VM
1	...	...	...	...	...	...	...	.5	...	N
..	1	...	...	...	...	...	...	.5	...	N
...	...	1	...	...	...	...	...	.5	...	AC
..	...	...	1	...	...	...	...	.5	...	AC
..	...	...	...	.5	...	...	...	.5	.5	N
..	...	...	...	...	.055	...	...	.5	.945	M
..	...	...	...	...	...	...	.4	.5	.6	N
..	...	...	...	.5	.055	...	.4	.5	.045	VM

In this experiment Normal brain and Fixed virus brain + rabid dog's serum showed some deviation of complement. The carbolized brains without the dog's serum showed slight absorption of complement. There was no greater absorption on the addition of the dog's serum.

.B	F.V.B	S.V.B G.P	R.D.S Dog.III	Dog Normal.	G.P.S	Sal.	Sheep S.	W.G.C	Sal.	Resul
1	...	...	...	...	.055	.245	.4	.5	.3	VM
1	...	...	...	...	.11	.19	.4	.5	.3	C
1	...	...	.5	...	.055	.245	.4	.5	.3	N
1	...	...	.5	...	.11	.19	.4	.5	.3	AC
1	...	...	...	.5	.055	.245	.4	.5	.3	T
1	...	...	...	.5	.11	.19	.4	.5	.3	C
..	1	...	...	...	.055	.245	.4	.5	.3	C
..	1	...	...	...	.11	.19	.4	.5	.3	C
..	1	...	.5	...	.055	.245	.4	.5	.3	NQC
..	1	...	.5	...	.11	.19	.4	.5	.3	C
..	1	...	...	.5	.055	.245	.4	.5	.3	C
..	1	...	...	.5	.11	.19	.4	.5	.3	C
1	...	...	...	...	...	...	...	.5	1.0	N
..	1	...	...	...	...	...	...	.5	1.0	N
..	...	...	...	.5	.055	...	.4	.5	.045	NQC
..	...	1	...	....	.055	.245	.4	.5	.3	C
..	...	1	...	...	.11	..19	.4	.5	.3	C
..	...	1	.5	...	.055	.245	.4	.5	.3	T
..	...	1	.5	...	.11	.19	.4	.5	.3	S
..	...	1	...	.5	.055	.245	.4	.5	.3	NQC
..	...	1	...	.5	.11	.19	.4	.5	.3	C
..	...	1	...	...	....	...	...	.5	.1	N
..	...	...	.5	...	.055	...	.4	.5	.045	VM
..	...	...	...	.5	...	...	...	.5	.5	N
..	...	...	...	...	.055	...	.4	.5	.545	C

In this experiment street virus was also used as antigen and normal dog serum was used as a control. With all the antigens used there was considerably more deviation of complement with the rabid dog's serum than with the normal dog's serum. There was most deviation with street virus brain, least with Fixed virus brain.

No.XIII.

N.B	F.V.B	S.V.B G.P	Dog III.	G.P.S	Sal.	Sheep S	W.G.C	Sal.	Result.
1	...	...	...	.055	.245	.4	.5	.3	S
1	...	...	...	.11	.19	.4	.5	.3	AC
1	...	...	.5	.055	.245	.4	.5	.3	N
1	...	...	.5	.11	.19	.4	.5	.3	T
...	1	...	...	.055	.245	.4	.5	.3	VM
...	1	...	...	.11	.19	.4	.5	.3	NQC
...	1	...	.5	.055	.245	.4	.5	.3	N
...	1	...	.5	.11	.19	.4	.5	.3	N
...	...	1	...	.055	.245	.4	.5	.3	S
...	...	1	...	.11	.19	.4	.5	.3	AC
...	...	1	.5	.055	.245	.4	.5	.3	N
...	...	1	.5	.11	.19	.4	.5	.3	N
...	...	...	.5	...	...	...	.5	.5	ST

This was a similar experiment to the last except that normal dog serum was not used as a control. The result showed a considerable deviation of complement with all the antigens used, i.e., normal brain, fixed virus brain and street virus brain. This time normal brain gave the least while Fixed virus brain and street virus brain gave the same amount of deviation.

N.B G.P	S.V.B G.P	Dog II	Dog III	Dog Normal.	G.P.S	Sal.	Sheep S	W.G.C.	Sal.	Result.
1	...	...	...	...	.055	.245	.4	.5	.3	VM
1	...	...	...	...	.11	.19	.4	.5	.3	NQC
1	...	.5	...	...	.055	.245	.4	.5	.3	N
1	...	.5	...	...	.11	.19	.4	.5	.3	N
1	...	...	.5	...	.055	.245	.4	.5	.3	N
1	...	...	.5	...	.11	.19	.4	.5	.3	N
1	...	...	...	.5	.055	.245	.4	.5	.3	N
1	...	...	...	.5	.11	.19	.4	.5	.3	S
...	1	...	...	...	.055	.245	.4	.5	.3	VM
...	1	...	...	...	.11	.19	.4	.5	.3	NQC
...	1	.5	...	...	.055	.245	.4	.5	.3	N
...	1	.5	...	...	.11	.19	.4	.5	.3	N
...	1	...	.5	...	.055	.245	.4	.5	.3	N
...	1	...	.5	...	.11	.19	.4	.5	.3	N
...	1	...	...	.5	.055	.245	.4	.5	.3	T
...	1	...	...	.5	.11	.19	.4	.5	.3	NQC
1	...	...	...	...	...	...	...	.5	...	N
...	...	.5	...	...	.055	...	.4	.5	.045	NQC
...	...	...	...	.5	.055	...	.4	.5	.045	C
...	...	...	...	...	.055	...	.4	.5	.4	C
...	...	.5	...	...	...	...	...	.5	.5	N
...	...	...	...	.5	.055	...	...	.5	.445	C

This experiment was controlled with normal dog serum: the antigens used being normal guinea-pig's brain and street virus guinea-pig's brain. The result was that rabid dog's serum + both antigens deviated more complement than did normal dog's serum. Rabid dog's serum alone absorbed a slight amount of complement while normal dog's serum did not. The rabid dog's serum + normal brain absorbed the same amount of complement as rabid dog's serum + street virus brain.

No. XV.

B P	S.V.B G.P	Dog II	Dog III	Dog Normal.	G.P.S	Sal.	Sheep S	W.G.C	Sal.	Result.
	...	...	...	...	.055	.245	.4	.5	.3	T
	...	...	...	...	.11	.19	.4	.5	.3	S
	...	.5	...	...	.055	.245	.4	.5	.3	N
	...	.5	...	...	.11	.19	.4	.5	.3	ST
	...	...	...	.5	.055	.245	.4	.5	.3	N
	...	...	...	.5	.11	.19	.4	.5	.3	T
.	1	...	...	...	.055	.245	.4	.5	.3	N
.	1	...	...	...	.11	.19	.4	.5	.3	S
.	1	.5	...	...	.055	.245	.4	.5	.3	N
.	1	.5	...	...	.11	.19	.4	.5	.3	T
.	1	...	...	.5	.055	.245	.4	.5	.3	N
.	1	...	...	.5	.11	.19	.4	.5	.3	ST
.	...	.2	...	....	.055	.4	...	.5	.445	VM
.	...	.4	...	...	.055	.2	...	.5	.445	VM
.	...	.6	...	...	.055	...	...	.5	.445	VM
.	...	...	...	.2	.055	.4	...	.5	.445	AC
.	...	...	...	.4	.055	.2	...	.5	.445	C
.	...	...	...	.6	.055	...	...	.5	.445	C

Similar to the last, but there was no appreciable difference between rabid dog's serum and normal dog's serum in the amount of deviation of complement produced. The antigen alone seemed to absorb as much complement as antigen + either rabid dog's serum or normal dog's serum.

No. XVI.

N.B	S.V.B G.P	Dog II	Dog III.	G.P.S	Sal.	Sheep S	W.G.C	Sal.	Result.
	1	...	...	.055	.245	.4	.5	.3	C
	1	...	...	.11	.19	.4	.5	.3	C
	1	...	.5	.055	.245	.4	.5	.3	N
	1	...	.5	.11	.19	.4	.5	.3	S
	1	...	.5	.055	...	.4	.5	...	S
	1	...	.5 not centrifuged	.11	...	.4	.5	...	NQC
	1	...	...	...	...	...	.5	...	N

In this experiment also street virus brain + Rabid dog's serum shows some deviation of complement.

No. XVII

E.S.M.G $\frac{1}{50}$	I.H.S 55	R.D.S 55	Saline.	G.P.S.	Sensitised Goat RBC.	Result.
1	.5	...	...	.025	.2	N
1	.5	...	...	.05	.2	N
1	.5	...	...	.075	.2	N
1	.5	...	...	.1	.2	T
1	...	.5	...	.025	.2	N
1	...	.5	...	.05	.2	T
1	...	.5	...	.075	.2	M
1	...	.5	...	.1	.2	VM
1	...	...	.5	.025	.2	M
1	...	...	.5	.05	.2	AC
1	...	...	.5	.075	.2	C
1	...	...	.5	.1	.2	Tube accidentally broken.

In this instance the antigen used was the submaxillary gland of a rabid dog. The immune sera used were the horse serum and a rabid dog's serum. The result shows a considerable amount of absorption of complement especially with the horse serum. Unfortunately controls could not be done either with normal serum or normal submaxillary gland.

SUMMARY AND CONCLUSIONS.

These experiments may be briefly summarized as follows:-

The results as regards complement fixation were inconstant using brain substance as antigen. A certain amount of fixation did occur, but it was never very marked. There were only very slight differences in the degree of the reaction with the various antigens used, e.g. normal brain gave a certain amount of deviation, though it was generally less than that given by the virus brains.

The brain antigen by itself appeared sometimes to absorb a certain amount of complement as did also to a less degree the horse and dog sera, but not to a sufficient extent to cause a misinterpretation of the results had the reaction been marked.

Of all the antigens used the best results were obtained with submaxillary gland emulsion which produced a very marked deviation of complement (Expt.No.18), but owing to the difficulty of obtaining material this experiment could not be properly controlled and for the same reason further work along these lines had to be abandoned for the present.

These results are very similar to those that have been obtained by other observers.

Heller and Tomarkin (3) using fixed virus brain as antigen found that complement was fixed to a certain extent, but that the same fixation took place with normal

brain. They found that with normal serum fixation was not produced.

Baroni, Cinca and Ionescu-Mihaiesti[4] got similar results. They found however that with the control sera the same amount of haemolysis occurred.

Redrigailoff and Saffchenko[5] got positive results using emulsions of submaxillary glands of dogs dead of street virus as antigen. They found no fixation with other rabic tissues or normal salivary glands.

Babes[6] got similar results using submaxillary gland emulsion as antigen.

All these observers used the sera of immunized animals and Babes considers that such deviation as is produced with brain antigens is due to the serum being specific for nerve substance, but these experiments show that a certain amount of deviation is also produced by rabid dogs serum with these antigens, so that there must be some degree of specificity in the reaction.

Since these experiments were performed Nogouchi[7] has claimed to have cultivated the virus of rabies and it is possible that in the future cultural methods may be used for the diagnosis of the disease, or that cultures may provide a suitable antigen for the complement fixation test.

The conclusion to be drawn from these experiments is that with the means at present at our disposal the complement deviation reaction is not sufficiently delicate or specific to be of assistance in the diagnosis of rabies, and they show that the most marked reaction is obtained by using a salivary gland emulsion as antigen.

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